Evaluation of time required for recontamination of coronally sealed canals medicated with calcium hydroxide and chlorhexidine

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Abstract

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Aim To determine *in vitro* the time required for recontamination of coronally sealed canals medicated with either calcium hydroxide ($Ca(OH)_2$), 2% chlorhexidine gel (CG) or with a combination of both.

Methodology Eighty intact, caries-free, premolar teeth with straight roots and mature apices were selected for the study. After biomechanical preparation of 75 teeth, they were randomly divided into nine groups according to the intracanal medicament and the coronal seal with 'Intermediate Restorative Material' (IRM) as follows: (i) 10 teeth medicated with CG, coronally unsealed; (ii) 10 teeth medicated with $Ca(OH)_2$, coronally unsealed; (iii) 10 teeth medicated with $Ca(OH)_2$ + CG, coronally unsealed; (iv) 10 teeth medicated with CG + coronal seal; (v) 10 teeth medicated with $Ca(OH)_2$ + coronal seal; (vi) 10 teeth medicated with CG + $Ca(OH)_2$ + coronal seal; (vii) 10 teeth without intracanal medicament and coronally sealed; (viii) 5 teeth without intracanal medicament and coronally unsealed, used as the positive control group (PC); (ix) 5 teeth with intact crowns used as the negative control group (NC). Glass flasks were filled with Brain Heart Infusion broth (BHI), so that only the root apex was in contact with the broth, while the crown was immersed in human saliva + BHI (3 : 1). The flasks were then incubated at 37 °C in an atmosphere of 10% CO_2, and microbial growth was checked daily.

Results All specimens of the PC showed contamination within 1 day of incubation, while the NC showed no evidence of broth turbidity. Recontamination was detected after an average time of 3.7 days in the unsealed canals medicated with CG, 1.8 days in the group medicated with $Ca(OH)_2$ and 2.6 days in the group medicated with $Ca(OH)_2$ + CG. When the crowns were sealed with IRM, recontamination was detected within 13.5 days in the canals medicated with CG, after 17.2 days in the group medicated with $Ca(OH)_2$ and after 11.9 days in the group medicated with CG + $Ca(OH)_2$. The group with no medication, but sealed with IRM, showed recontamination after 8.7 days. There were statistically significant differences between the teeth with or without coronal seal ($P < 0.05$).

Conclusion The coronal seal delayed but did not prevent leakage of microorganisms. There was no difference between the various medicaments.

Keywords: antimicrobial activity, calcium hydroxide, chlorhexidine, endodontics, intracanal medicament, IRM.

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Introduction

Bacteria and their by-products are the main causes of pulpitis and apical periodontitis (Kakehashi *et al.* 1965, Gomes *et al.* 1994, 1996a,b,c, Estrela *et al.* 1998). The outcome of root canal treatment depends on the reduction or elimination of bacteria present within the tooth and thus on the effectiveness of chemomechanical preparation (Siqueira & Uzeda 1997).

The complex anatomy of root canals provides a supportive environment for growth, multiplication and interaction of microorganisms in pulpal infections (Biffi & Rodrigues 1989). Difficulties in antimicrobial control require the use of intracanal dressings that complement chemomechanical preparation, especially in the

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presence of pain and/or persistent exudate (Estrela *et al.* 1998).

Intracanal medicaments should have a broad antibacterial spectrum, no cytotoxicity, and should possess physicochemical properties that permit diffusion through the dentinal tubules and lateral ramifications of the root canal system (Alencar *et al.* 1997).

Calcium hydroxide ($Ca(OH)_2$) is considered to possess many properties of an ideal root canal dressing (Beltes *et al.* 1997), and has become popular because of its antimicrobial and biological properties (Estrela *et al.* 1998). The antimicrobial action of $Ca(OH)_2$ is related to its ionic dissociation into calcium and hydroxyl ions and their toxic effects on bacteria, by inhibiting cytoplasmic membrane enzymes with consequent changes in the organic components and in nutrient transport (Estrela *et al.* 1998).

Calcium hydroxide-containing materials have been used widely in endodontic therapy to stimulate apexification, repair perforations, promote healing by hard tissue formation in horizontal and vertical root fractures and control external and internal inflammatory root resorption (Beltes *et al.* 1997). $Ca(OH)_2$ also mediates the neutralisation of lipopolysaccharides (Safavi & Nichols 1994), and thus helps in cleansing the root canal (Hasselgren *et al.* 1988). However, $Ca(OH)_2$ cannot be considered as a universal intracanal medicament, as it is not equally effective against all bacteria found in the root canal (Gomes *et al.* 2002). Indeed, several studies have reported the failure of $Ca(OH)_2$ to eliminate enterococci effectively (Engström 1964, Cavalleri *et al.* 1989, Gomes *et al.* 1996c, Molander *et al.* 1998, Pinheiro *et al.* 2003), as they tolerate high pH values, varying from 9 to 11.

Chlorhexidine gluconate has been widely used in periodontics because of its antibacterial activity (Gjermo 1974, Lindskog *et al.* 1998). Its use in endodontics has been proposed as both an irrigant and an intracanal medicament (Delany *et al.* 1982, Vahdaty *et al.* 1993, Jeansonne & White 1994, Siqueira & Uzeda 1997, Ferraz *et al.* 2001, Gomes *et al.* 2001). It has been demonstrated that chlorhexidine has inhibitory effects on bacteria commonly found in endodontic infections (Cervone *et al.* 1990). Chlorhexidine has antimicrobial activity against Gram-positive and Gram-negative microorganisms (Waaler 1990). Its efficacy is based on the interaction between the positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall, which allows the chlorhexidine molecule to penetrate the bacteria with intracellular toxic effects (Hugo & Longworth 1964, Lindskog *et al.* 1998).

The aim of combining $Ca(OH)_2$ and 2% chlorhexidine gel (CG) is to enhance antimicrobial effectiveness, particularly against resistant microorganisms such as *Enterococcus faecalis* that are implicated in the failure of root canal treatment (Engström 1964, Cavalleri *et al.* 1989, Gomes *et al.* 1996b, Molander *et al.* 1998, Peciuliene *et al.* 2000, Pinheiro *et al.* 2003). The association of CG and $Ca(OH)_2$ has been tested against *E. faecalis* in infected bovine root dentine, demonstrating that the combined medicaments were effective (Almyroud *et al.* 2002, Gomes *et al.* 2003).

The objective of this study was to determine *in vitro* the time required for recontamination of canals medicated with either $Ca(OH)_2$, CG or a combination of both.

Materials and methods

Eighty intact, caries-free, human premolar teeth with straight roots were selected for the study. The teeth were autoclaved and kept in 0.5% sodium hypochlorite (NaOCl) for no longer than 7 days. Conventional access preparations were made in 75 teeth, and the cervical two-thirds of the canals were prepared initially using files up to size 35. They were then flared with a size 2 Gates-Glidden bur (GG), followed by a size 3 bur, 1 mm shorter. A size 10 K-file was introduced into each canal until it appeared at the apical foramen. The working length was established by subtracting 1 mm from this measurement. The same K-file was used to recapitulate the canal 1 mm beyond its length between each file in order to preserve patency. The root canals were prepared using the step-back technique with K-files manipulated in a reaming motion. To standardise the diameter, the apical foramen was enlarged to a size 20 K-file. Apical preparation was performed at the working length up to a size 35 K-file and completed by stepping back at 1-mm increments. Irrigation was carried out using 1 mL of a 2.5% NaOCl solution between files. After preparation, the root canal was irrigated with 5 mL 17% EDTA for 3 min to remove the smear layer, and then with 1 mL 5.25% NaOCl. Five millilitres of sterile saline was used as a final rinse to remove debris and the irrigants.

The teeth were divided randomly according to the intracanal medicament and coronal seal with 'Intermediate Restorative Material' (IRM; LD Caulk Division, Milford, DE, USA), as follows:

- Group 1: 10 teeth with CG, unsealed coronally;
- Group 2: 10 teeth with $Ca(OH)_2$, unsealed coronally;
- Group 3: 10 teeth with CG + $Ca(OH)_2$, unsealed coronally;
- Group 4: 10 teeth with CG + coronal seal;
- Group 5: 10 teeth with $Ca(OH)_2$ + coronal seal;

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- Group 6: 10 teeth with CG + $Ca(OH)_2$ (1 : 1) + coronal seal;
- Group 7: 10 teeth without intracanal medication + coronal seal;
- Group 8: 5 teeth used as the positive control group (PC), without intracanal medication and coronally unsealed;
- Group 9: 5 teeth used as the negative control group (NC), with intact crowns.

The chlorhexidine gel consisted of gel base (1% natrosol) and chlorhexidine gluconate, and was prepared in a standard way (Drogal Farmácia de Manipulação Ltda., Piracicaba, SP, Brazil). The natrosol gel (hydroxyethyl cellulose, pH 5.5), used as the base, is soluble in water and widely used to thicken shampoos, gels and soaps. The gel formulation may keep the 'active principle' of chlorhexidine gluconate in contact with the microorganisms for inhibiting growth for a longer period (Gomes *et al.* 2001).

The $Ca(OH)_2$ paste was prepared from $Ca(OH)_2$ powder (Quimis Mallinkrodt, Inc., Phillipsburg, NJ, USA), using polyethyleneglycol (Merck, Darmstadt, Germany) as a vehicle (2 : 1), in order to facilitate its placement with a syringe inside the root canals. The consistency of the paste was similar to that of a toothpaste with a viscosity of 3501 cP at 0.1 r.p.m. (Brookfield Digital Reometer, model DV-III-IV, São Paulo, SP, Brazil).

The pH of the chlorhexidine and the $Ca(OH)_2$ pastes, alone and combined, was also tested (Procyon, digital pH meter model AS 720, electrode A 11489, Procy Instrumental Científica, São Paulo, SP, Brazil).

The apparatus used to evaluate leakage was prepared as previously described by Siqueira *et al.* (1999) (Fig. 1). Glass vials with rubber stoppers were adjusted for use. Using a high-speed handpiece, a hole was made through the centre of each rubber stopper (Torabinejad *et al.* 1990) in which each tooth was inserted under pressure up to its cemento-enamel junction, so that its crown was outside the vial and its root was within.

Cylinders prepared from 10-mL plastic syringes were adapted to the outer surface of the stoppers to create a chamber around the crown of the tooth.

The glass flasks were then filled with Brain Heart Infusion broth (BHI; Oxoid, Basingstoke, UK) plus neutralisers so that a 2-mm length of root apex was immersed in the broth. The neutralisers were used to prevent continued action of the medication if it reached the BHI medium through the apical foramen. The neutraliser for $Ca(OH)_2$ was 0.5% citric acid, while 0.5% Tween 80 + 0.07% lecithin was used for chlorhexidine alone or combined with $Ca(OH)_2$ (Siqueira *et al.* 1998).

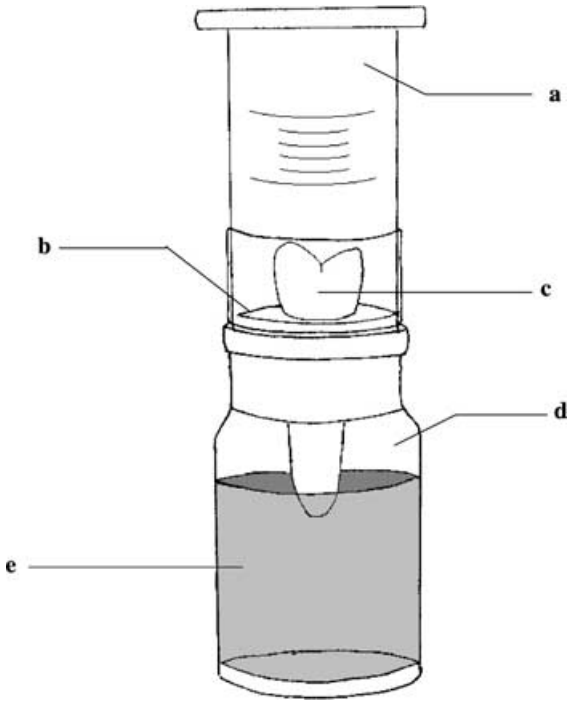

Figure 1 Apparatus used to assess the root canal system reinfection. (a) Syringe (saliva reservoir); (b) size 1 rubber stopper; (c) tooth; (d) 10-mL vial; (e) Brain Heart Infusion (BHI) medium.

The flasks containing BHI, the stoppers with the teeth and the syringes were separately autoclaved at 121 °C for 15 min, and then adapted to the flasks + stoppers under a laminar flow hood. Cyanocrylate was then applied to the interface between the tooth and the stopper to avoid saliva penetration into the BHI broth (Imura *et al.* 1997). In order to ensure the efficiency of the cyanocrylate seal, 2 mL of 1% sterile methylene blue dye was placed into the tube leading to the coronal portion of each sample (Malone & Donnelly 1997). If the medium became blue, this meant the seal was defective and the specimen was discarded. Parafilm® (American National Can™, Menasha, WI, USA) was used to block the interface between flasks and stopper. The flasks were then incubated at 37 °C for 4 days to ensure sterilisation.

After the fourth day, the syringe chambers were removed so that the methylene blue could be removed with plastic pipettes. The medicaments were prepared and injected into the root canals with sterile plastic syringes. In order to check the correct placement of the medication inside the canals, buccal–lingual and mesial–distal radiographs were taken, and the passage of a small amount of medication through the apical foramen was permitted.

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The teeth from groups 4–7 had their coronal access filled with IRM, a reinforced zinc oxide–eugenol-based temporary restorative material, in order to avoid saliva penetration into the canals.

The depth of the cavity was measured from the canal orifice to the cavosurface margin with a periodontal probe. All samples allowed for approximately 4 mm of the restorative material to comply with the recommendation of Webber *et al.* (1978). To verify the uniformity and density, all teeth were radiographed buccal–lingually and mesial–distally after the placement of IRM.

Human whole saliva (30 mL) was collected from one individual at 8 AM on each day of exposure or solution change. The volunteer did not brush or floss for at least 12 h before collection. Chewing a 1-g piece of Parafilm® (American National Can™, Menasha,WI, USA) was used to stimulate salivary flow. The saliva was stored in a brown glass, 100 mL screw-top container, according to Magura *et al.* (1991). The chamber of each whole apparatus was put in place again and filled with 3 mL of human saliva and BHI broth in a 3 : 1 proportion. The mixture was replaced every 3 days.

The whole apparatus was incubated at 37 °C and checked daily for the appearance of turbidity in the BHI broth. As the medium can evaporate, care was also taken to ensure that the roots remained in contact with the BHI medium throughout the experiment.

All data were organised in a contingency table, and the Kruskal–Wallis test was applied for statistical analysis, with the level of significance set at 5% ($P < 0.05$).

Results

The average time for the complete reinfection of the root canal system is shown in Table 1. All specimens of the PC showed broth turbidity within 1 day of incubation. On the other hand, there was no evidence of broth turbidity in the NC throughout the experiment.

The canals without coronal seal, but medicated with CG, showed recontamination after an average time of 3.7 days; the group with $Ca(OH)_2$ after 1.8 days and the group with CG + $Ca(OH)_2$ after 2.6 days.

The canals medicated with CG + IRM showed recontamination within 13.5 days; the group with $Ca(OH)_2$ + IRM after 17.2 days and the group with CG + $Ca(OH)_2$ + IRM after 11.9 days.

The group with no medication, but sealed with IRM, showed recontamination after an average time of 8.7 days.

There were statistically significant differences between the groups ($P < 0.05$). All groups without coronal seal were recontaminated significantly more quickly than those sealed with IRM, except those teeth coronally sealed but without medicament. The groups with intracanal medication and sealed were not significantly different from each other. The pH of chlorhexidine was 7.0, calcium hydroxide paste was 11.0 and chlorhexidine + calcium hydroxide was 12.8.

Table 1 Number and average days to complete recontamination of root canals after saliva challenge

Samples	CG	$Ca(OH)_2$	CG + $Ca(OH)_2$	CG + IRM	$Ca(OH)_2$ + IRM	CG + $Ca(OH)_2$ + IRM	IRM	PC	NC
1	3	3	2	14	20	15	5	1	No bacterial growth
2	3	1	2	11	3	15	6	1	No bacterial growth
3	5	1	2	21	20	4	17	1	No bacterial growth
4	4	1	3	22	20	13	15	1	No bacterial growth
5	4	3	3	5	27	13	5	1	No bacterial growth
6	1	1	3	21	3	20	7	–	–
7	4	2	3	5	27	4	7	–	–
8	4	1	3	14	20	10	15	–	–
9	5	2	2	11	7	18	5	–	–
10	4	3	3	11	25	7	5	–	–
Average days ± SD	**3.7** ± 1.16a	**1.8** ± 0.92a	**2.6** ± 0.52a	**13.5** ± 6.22b,c	**17.2** ± 9.38c	**11.9** ± 5.55b,c	**8.7** ± 4.9a,b	**1** ± 0.0a	–

CG: 2% chlorhexidine gel; PC: positive control group; NC: negative control group.

SD: Standard deviation.

Different letters mean significant statistical difference ($P < 0.05$).

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Discussion

One of the objectives of using an intracanal medicament is to reduce the number of microorganisms. Ideally, it should penetrate areas not reached by the instruments or irrigating solution during root canal preparation (Barbosa *et al.* 1997).

The methodology used in this study was similar to that reported previously (Imura *et al.* 1997, Malone & Donnelly 1997, Siqueira *et al.* 1999). The placement of medicaments inside the canals and the coronal seal was checked radiographically (except those with chlorhexidine as this medication is radiolucent). Pilot studies demonstrated that the presence of neutralisers in 8-mL BHI broth allowed bacterial growth, even when 200 μL of the medicaments was added. Furthermore, undesirable saline penetration into the BHI broth (Imura *et al.* 1997) as a result of a leakage along the interface between the tooth and the stopper was visually checked by means of sterile 1% methylene blue dye. Care was also taken to ensure that the roots were in contact with the BHI medium throughout the experiment, in order to avoid false negative results.

It was also observed that the groups medicated and unsealed, and the groups unmedicated and sealed did not show statistically significant difference ($P > 0.05$) from the PC. Therefore, the association of an intracanal medicament and the coronal seal is essential to prevent the reinfection of the root canal system between appointments, as demonstrated in previous studies (Imura *et al.* 1997, Malone & Donnelly 1997, Siqueira *et al.* 1999).

During root canal treatment, it is important to create a seal in the access cavity in order to prevent the entrance of saliva and microorganisms into the root canal system and the escape of the intracanal medicaments into the oral cavity. Even in root-filled teeth, if the coronal restoration becomes defective or is lost, the eventual recontamination of the root canal system can result in failure of endodontic treatment (Malone & Donnelly 1997, Zaia *et al.* 2002).

Even though IRM delayed the entrance of saliva and microorganisms into the root canal system by up to 4 days, it did not prevent microleakage, agreeing with the findings of Costas & Wong (1991) and Zaia *et al.* (2002), and justifying research efforts to incorporate dentine-bonding agents and resin as sealers (Leonard *et al.* 1996, Zaia *et al.* 2002). Further long-term evaluations are indicated.

The similar behaviour of the three intracanal medicaments tested, in the presence or absence of a coronal seal, may be explained by the fact that their main function in this study was to act as a barrier against coronal microleakage, as the samples had been previously autoclaved. On the other hand, in infected root canals, a medicament should also reach and be effective against selected endodontic microorganisms inside the dentinal tubules and ramifications of the root canal system. In this context, the tested medicaments may present different behaviour, as they will also depend upon the vulnerability of the involved species, which may not be uniform (Gomes *et al.* 1996c).

Conclusion

The coronal seal delays coronal microleakage, as does the use of an intracanal medicament.

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